

Butyrate and retinoic acid imprint mucosal-like dendritic cell development synergistically from bone marrow cells

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Introduction

It is well known that intestinal mucosa is the primary breeding place for flora and various foreign antigens. Thus, it is necessary for the mucosal immune system to create a protective immune response or to maintain tolerance [1]. As with antigen-presenting cells, dendritic cells are virtually omnipresent and play a pivotal role in instructing the initiation and activation and controlling the effects of the antigen-induced immune response in peripheral immune organs, as well as immune tolerance in the gut [2]. Dendritic cells (DCs) are remarkably plastic and have a notable ability to adapt to the resident microenvironment through modification of their phenotypes and functions [3]. Since the discovery of DCs, various DC subsets, which show dramatically different phenotypes and immune functions, have been identified [4,5]. In mucosal tissue there are at

Summary

Accumulating data show that the phenotypes and functions of distinctive mucosal dendritic cells (DCs) in the gut are regulated by retinoic acid (RA). Unfortunately, the exact role of butyrate in RA-mediated mucosal DC differentiation has not been elucidated thoroughly to date. Mucosal-like dendritic cell differentiation was completed *in vitro* by culturing bone marrow cells with growth factors [granulocyte-macrophage colony-stimulating factor (GM-CSF/interleukin (IL)-4), RA and/or butyrate. The phenotypes, cytokine secretion, immune functions and levels of retinal dehydrogenase of different DCs were detected using quantitative polymerase chain reaction (qPCR), enzyme-linked immunosorbent assay (ELISA) and flow cytometry, respectively. The results showed that RA-induced DCs (RA-DCs) showed mucosal DC properties, including expression of CD103 and gut homing receptor $\alpha_4\beta_7$, low proinflammatory cytokine secretion and low priming capability to antigen-specific CD4⁺ T cells. Butyrate-treated RA-DCs (Bu-RA-DCs) decreased CD11c, but increased CD103 and $\alpha_4\beta_7$ expression. Moreover, the CD4⁺ T priming capability and the levels of retinal dehydrogenase of RA-DCs were suppressed significantly by butyrate. Thus, butyrate and retinoic acid have different but synergistic regulatory functions on mucosal DC differentiation, indicating that immune homeostasis in the gut depends largely upon RA and butyrate to imprint different mucosal DC subsets, both individually and collectively.

Keywords: butyrate, dendritic cell, retinoic acid, short chain fatty acid, T cell

least two different DC subsets, depending on the expression of CD103, and these DCs decide gut tolerance and intestinal inflammation [6,7]. Accumulating data have shown that metabolites or products derived from commensal bacteria have contributed greatly to the development, homeostasis and properties of DCs in the mammalian gastrointestinal tract [8,9]. If the composition or metabolism of the microflora is altered, inflammatory diseases such as inflammatory bowel disease (IBD), atherosclerosis and even colon cancer might be induced [10]. Thus, these distinctive DC subsets and maintenance of a tolerant gut immune microenvironment depend largely upon the interaction between DCs and commensal bacterial populations.

In recent decades, studies have shown that vitamin A-derived retinoic acid (RA) plays vital roles in maintaining gut immune homeostasis through the induction of gut homing in lymphocytes and the differentiation of

regulatory T cells (T_{reg}) [11]. It also has been shown that RA has the capability to promote the development of mucosal DCs, including intestinal $CD103^+CD11b^-(cDC1)$ and $CD103^+CD11b^+(cDC2)$ cells [9]. In addition to RA, the fates of intestinal immune cells in the gut are also decided by many other factors, especially signals from commensal bacteria and their metabolites, such as short chain fatty acids (SCFAs) [12]. As one of the richest SCFAs in the gut, butyrate is produced by intestinal bacteria through the fermentation of plant fibre, and functions in inducing the differentiation of T_{reg} cells [13]. Furthermore, studies have shown that lower levels of butyrate were detected in the lumen of patients with colitis, indicating a potential function of butyrate in regulating immune function in the gut [14]. Unfortunately, at present, the exact role of butyrate in the differentiation of RA-induced mucosal DCs is still not well demonstrated. In this study, by co-culturing butyrate with RA to imprint bone marrow cell differentiation into DCs *in vitro*, we observed that butyrate co-operates with RA to induce mucosal-like DC differentiation. These results indicate a potential contribution of butyrate in maintaining gut immunity with tolerance properties.

Materials and methods

Mice

C57BL/6 mice (aged 6–12 weeks) were purchased from the animal facility of the Medical Center at Yangzhou University, China. Ovalbumin (OVA)_{323–339} peptide-specific T cell receptor (TCR) transgenic mice DO11.10 (BALB/c background) and forkhead box protein 3 green fluorescent protein (FoxP3^{GFP}) mice (B6.Cg-FoxP3^{tm1Mal}/J) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). To prepare C57BL/6 × DO11.10 F1 mice, male C57BL/6 mice were crossed with female DO11.10 mice. Similarly, DO11.10 × FoxP3^{GFP} F1 mice were generated by crossing female DO11.10 mice with male FoxP3^{GFP} mice. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Jiangsu University in China.

Dendritic cells prepared with granulocyte–macrophage colony-stimulating factor (GM-CSF)/interleukin (IL)-4 and RA or butyrate *in vitro*

Bone marrow-derived dendritic cells were prepared by culturing bone marrow cells with GM-CSF (10 ng/ml) and IL-4 (1 ng/ml) (PeproTech, Rocky Hill, NJ, USA). To induce RA-DC, Bu-DC or RA-Bu-DC differentiation, RA (1 μ M) (Sigma-Aldrich, St Louis, MO, USA) and/or butyrate (0.5 mM) (Sigma-Aldrich), respectively, were added into DC culture wells beginning at day 3. In some experiments, bone marrow cells were first labelled with carboxyfluorescein succinimidyl ester (CFSE) using the CellTraceTM CFSE Cell Proliferation Kit (Invitrogen, Carlsbad, CA, USA). DC cells were purified with

anti-CD11c magnetic beads (Stemcell Technologies Inc., Vancouver, Canada), according to the manufacturer's protocol.

Antibody and flow cytometer analysis

All fluorescein-labelled antibodies [CD4, CD25, CD11c, CD11b, CD80, CD86, major histocompatibility complex (MHC)-II, B7-DC, CD103, Toll-like receptor (TLR)-4 and $\alpha_4\beta_7$] and 7-aminoactinomycin D (7-AAD) were purchased from eBioscience (San Diego, CA, USA). Cells were collected and incubated with rat serum to block Fc receptors, then incubated with fluorescein-labelled antibodies diluted to optimal concentrations for 20 min at 4°C. After being washed with phosphate-buffered saline (PBS), cells were then analysed using a fluorescence activated cell sorter (FACS) Calibur flow cytometer or Accuri C6 Flow Cytometer (BD Biosciences, San Jose, CA, USA). All flow cytometer data were analysed using Accuri C6 analysis software or FlowJo software (Tree Star Inc., Ashland, OR, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from bone marrow dendritic cells (BMDCs) with TRIzol reagent, followed by cDNA synthesis with Superscript Reverse Transcriptase (ThermoScientific, Waltham, MA, USA). Quantitative polymerase chain reactions (PCR) were performed using fluorochrome SYBR Green Premix Ex TaqTM (Takara, Dalian, Liaoning, China) on a Bio-Rad iCycler. Primers were synthesized by the Sangon Biotech Company (Shanghai, China). Total amounts of DNA were calculated using the $2^{-\Delta\Delta C_t}$ method, and the relative mRNA levels of target genes were normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The sequences of primers were as follows: *GAPDH*, forward 5'-GGCATGCTCTCAATGACAA-3', reverse 5'-TGTGAGGGA GATGCTCAGTG-3'; *IL-6*, forward 5'-GAGGAGACTTCA CAGAGGATAC-3', reverse 5'-GACTCTGGCTTTGTCTTT CTTG-3'; *IL-12*, forward 5'-GGTTTGCCATCGTTTG-3', reverse 5'-GGGTCTTTCCAGAGCCT-3'; *TNF- α* , forward 5'-GAAGTGGCAGAAGAGGCACT-3', reverse 5'-GGTCT GGGCCATAGAACTGA-3'; *Nos2*, forward 5'-CCAAGCCCT CACCTACTTCC-3', reverse 5'-CTCTGAGGGCTGACACAA GG-3'; *ALDH1A2*, forward 5'-ACCGTGTCTCCCAA CGTCACTGAT-3', reverse 5'-TGCAATTGCGGAGGATACCA TGAGA-3'.

Griess reaction

The concentration of nitric oxide (NO) in samples was determined using the Griess reaction kit (Beyotime, Nanjing, China), according to the manufacturer's protocol. The NO concentrations in samples were determined quantitatively based on their correspondence to the absorbance from the standard plot.

Aldehyde dehydrogenase (ALDH) activity assay

ALDH activity in cells was determined by using the ALDEFLUOR staining kit (StemCell Technologies), according to the manufacturer's instructions. ALDEFLUOR-stained cells were analysed using the FACSCalibur flow cytometer.

Antigen-specific CD4⁺ T cell proliferation assay *in vitro*

DC cells were prepared as described previously and incubated with 1 mg/ml OVA_{323–339} peptide (Sigma-Aldrich) at 37°C in a 5% CO₂ incubator for more than 6 h to prepare OVA_{323–339}-loaded DCs. OVA_{323–339} peptide-specific CD4⁺ T cells were collected from the spleens of C57BL/6 × DO11.10 F1 mice and purified using a CD4⁺ T cell selection kit (Stem Cell Technologies), according to the manufacturer's instructions. To induce CD4⁺ T cell proliferation, DCs (2 × 10⁴) and CFSE-labelled CD4⁺ T cells (2 × 10⁵) were seeded into the wells of round-bottomed 96-well plates, and then co-cultured in 5% CO₂ at 37°C. On day 5, cells were collected and washed with PBS, and then incubated with phycoerythrin (PE)-conjugated CD4 antibody and 7-AAD (eBioscience, San Diego, CA, USA). The dilutions of CFSE in 7-AAD-negative CD4⁺ T cells were analysed using the FACSCalibur flow cytometer.

FoxP3⁺ regulatory cell differentiation *in vitro*

Naive CD4⁺ T cells in the splenocytes of C57BL/6 × FoxP3^{GFP} F₁ mice were purified using EasySepTM mouse naive CD4⁺ T cell isolation kit (Stem Cell Technologies), according to the manufacturer's protocol. To induce FoxP3⁺ T_{regs} differentiation *in vitro*, DC cells (2 × 10⁴) and naive CD4⁺ T cells (2 × 10⁵) were seeded into a round-bottomed 96-well plate in 200 µl medium, then co-cultured further in 5% CO₂ at 37°C for 4 days. On day 5, cells were harvested and incubated with fluorescein-conjugated CD4 and CD25 antibodies and analysed by flow cytometry.

ELISA analysis for cytokines

The concentrations of IL-2 or IL-12p70 in the supernatant were determined, respectively, using an enzyme-linked immunosorbent assay (ELISA) kit (eBioscience), according to the manufacturer's protocol.

Statistical analysis

The experimental data are presented as the mean ± standard error of the mean (s.e.m.). The statistical analysis was performed using one-way analysis of variance (ANOVA) in Graphpad Prism version 5; *P* < 0.05 was considered to be statistically significant.

Results

Butyrate modifies the phenotype of RA-DCs

BM cells were cultured with GM-CSF/IL-4 with or without Bu and RA. As shown in Fig. 1a–c, there was no significant difference in the total cell numbers, cell size or cell divisions in different DCs. CD11c is one important biomarker of dendritic cells [15]. Comparing BMDCs and RA-DC cells, the expression of CD11c on Bu-DC (GM-CSF/IL-4/Bu) and RA-Bu-DC (GM-CSF/IL-4/RA/Bu) was inhibited dramatically (Fig. 1d). Both co-stimulatory molecules and MHC-II molecules play important roles in DC-mediated antigen presentation and T cell activation. The data in Fig. 2a show that the levels of CD80, CD86, MHC-II and B7-DC on DCs were obviously inhibited in RA-DCs. CD103 is a biomarker of a proportion of mucosal DCs (CD103⁺DC) in mesenteric lymph nodes (MLNs) and colonic lamina propria (LP). The results in Fig. 2a show that RA treatment increased the level of CD103 in DCs (RA-DC) significantly while butyrate had no effects, similar to DC treatment with RA. If DCs were treated with retinoic acid and butyrate simultaneously, higher CD103 were detected on DCs (RA-Bu-DC) (Fig. 2a). Interestingly, butyrate was found to have synergistic effects with RA in terms of the expression of gut homing receptor α₄β₇ in DCs (Fig. 2b).

Lower proinflammatory cytokines secreted by butyrate and RA-induced DCs

Next, we investigated the effects of butyrate and retinoic acid on the cytokine profile of DCs. The data in Fig. 3a,b,d,e show that both butyrate and retinoic acid treatment suppressed the expression of IL-6, tumour necrosis factor (TNF)-α and IL-12 in DCs (RA-DC, Bu-DC and RA-Bu-DC), which was detected using qPCR and ELISA. Interestingly, lipopolysaccharide (LPS)-treated BMDCs up-regulated nitric oxide synthase 2 (NOS2) levels and produced more NO, but these compounds were inhibited significantly in all RA and/or butyrate treated DCs (Fig. 3c,f). Thus, lower levels of proinflammatory cytokine secretion were detected in RA- and/or butyrate-treated DCs.

Butyrate regulates the priming functions of RA-DCs on CD4⁺ T cells

A hallmark of DCs is their ability to stimulate antigen-restricted T cell activation and proliferation. Comparing the priming capabilities of different DCs in Fig. 4a, we found that RA-treated DCs (RA-DCs) showed a decreased ability to prime CD4⁺ T cell proliferation than DCs/LPS. Remarkably, the proliferations of CD4⁺ T cells were suppressed more significantly in both Bu-DCs and RA-Bu-DCs compared to RA-DCs (Fig. 4a). This was confirmed further by detecting the levels of IL-2 in the supernatant of DC/CD4⁺ T cells culture wells (Fig. 4b). We next assessed the function

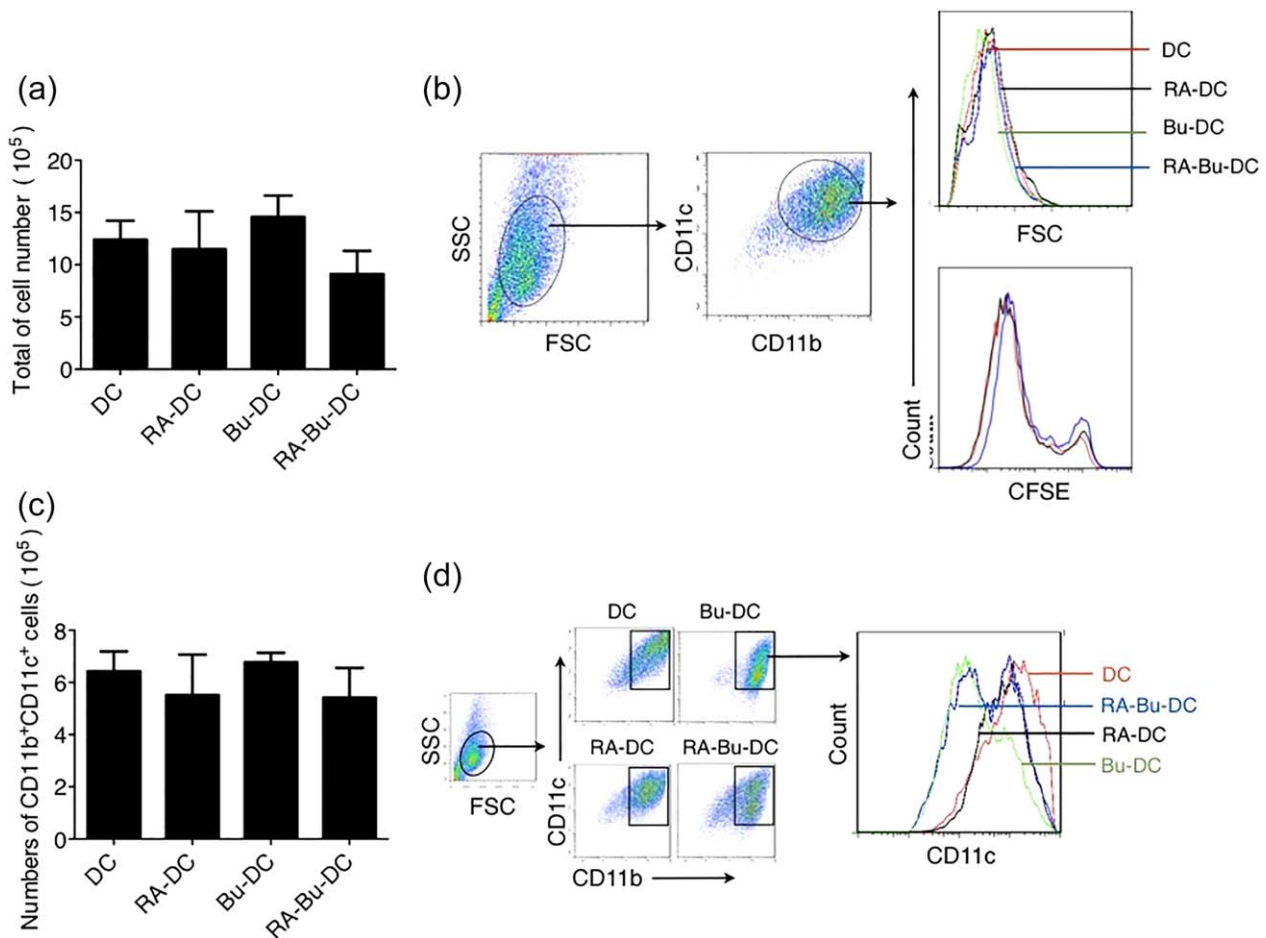


Fig. 1. Butyrate suppresses CD11c expression in bone marrow differentiated dendritic cells (DCs). Bone marrow cells were labelled with carboxyfluorescein succinimidyl ester (CFSE) and then cultured with granulocyte-macrophage colony-stimulating factor/interleukin 4 (GM-CSF/IL-4) with or without retinoic acid (RA) or butyrate (Bu) for 7 days to induced DC cell differentiation. On day 8, all cells were collected and total cell number (a), forward-scatter (FSC) channel and CFSE dilution of cells in each well (b), total numbers of CD11b⁺CD11c⁺ DC cells (c) and CD11c expression (d) of DCs were analysed. FSC in the graph indicates cell size and CFSE dilution indicates cell proliferation or division. DC, cultured with GM-CSF/IL-4; RA-DC, cultured with GM/IL-4/RA; Bu-DC, cultured with GM-CSF/IL-4/butyrate; RA-Bu-DC, cultured with GM-CSF/IL-4/RA/butyrate. Data are shown as the mean \pm standard error of the mean. [Colour figure can be viewed at wileyonlinelibrary.com].

of RA and/or butyrate on regulatory T cell differentiation *in vitro*. The data in Fig. 4c show that RA treatment obviously promoted CD4⁺ T_{reg} cell differentiation. Unfortunately, fewer FoxP3⁺ T cells were found in butyrate-treated groups, indicating that butyrate itself displays inefficient regulation of the differentiation of CD4⁺ regulatory T cells. Furthermore, the data in Fig. 4d show lower levels of IL-2 in RA-DCs and RA-Bu-DCs, indicating that T_{reg} cells consumed more IL-2 proteins in these groups.

Butyrate inhibits the activity of retinal dehydrogenase in RA-DCs

The metabolism of retinoic acid depends largely upon the function of members of the retinal dehydrogenase family, especially ALDH1a2. The data in Fig. 4e show that ALDH1a2 could be detected in the BMDCs and increased

further in response to LPS stimulation. Similar to previously reported data, RA treatment increased the ALDH1a2 activity in RA-DCs. It is worth noting that the activities of ALDH1a2 were suppressed dramatically in both groups of butyrate treated DCs (Bu-DC and RA-Bu-DC).

Discussion

The exact effect of butyrate on the differentiation of RA-induced mucosal DCs has not been elucidated thoroughly to date. In this study, we observed that butyrate treatment suppressed the expression of co-stimulatory MHC-II molecules but increased the levels of CD103 and $\alpha_4\beta_7$ in RA-induced DCs (RA-Bu-DCs). Moreover, the decreased capability of RA-DCs to prime OVA_{323–339} peptide antigen-specific CD4⁺ T cell proliferation was inhibited by

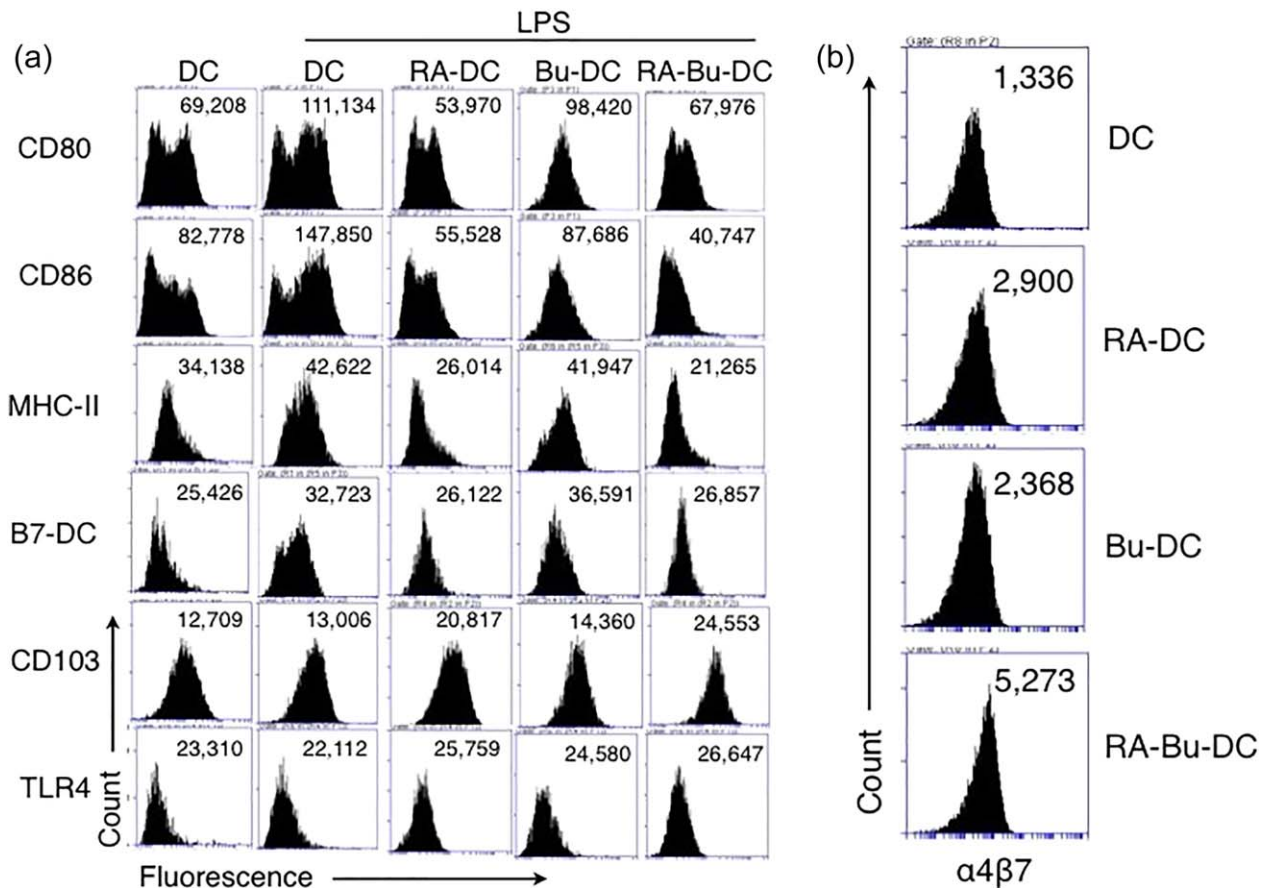


Fig. 2. Butyrate promotes retinoic acid (RA)-induced dendritic cells (DCs) to express CD103 and $\alpha_4\beta_7$ on the cell membrane. DC cells were prepared as in Fig. 1, and 1 $\mu\text{g}/\text{ml}$ lipopolysaccharide (LPS) was added to purified cells on day 7 to induce DC cell maturation. The next day, cells were harvested to analyse the expression of CD80, CD86, major histocompatibility complex (MHC)-II, B7-DC, CD103 and Toll-like receptor (TLR)-4 (a) and $\alpha_4\beta_7$ (b) using a flow cytometer. Numbers in the graphs are shown as the mean fluorescence intensities. [Colour figure can be viewed at wileyonlinelibrary.com].

butyrate. Thus, this study shows that butyrate had synergistic functions with RA to induce mucosal-like DC differentiation from bone marrow cells *in vitro*.

DCs work as the conductor in deciding the results of an immune response. As one of the DC subsets, CD103⁺ DCs represent the DC population in the intestinal LP and the MLN, and play an important role in mediating immunological tolerance in the gut [16,17]. This is largely dependent upon the relationship between the flexibility of CD103⁺ DCs and gut microenvironment. Evidence shows that retinoic acid induces DC cells to express $\alpha_4\beta_7$, and confers mucosal DC properties on them [9,18,19]. These data indicate the direct effects of retinoic acid on the differentiation of gut-resident DC cells. Here, we found that RA treatment promoted BMDC differentiation with mucosal-like DC properties. RA-treated cells express higher CD103 and $\alpha_4\beta_7$ expression, but decreased the ability to induce antigen CD4⁺T cell proliferation (Fig. 4). Indeed, the positive expression of CD103 and $\alpha_4\beta_7$ indicate that these RA-DCs were endowed with the correct set of homing receptors for

subsequent entry into mucosal effector tissues. Moreover, these RA-DCs have a reduction in the production of proinflammatory cytokines (TNF- α , IL-6) and IL-12 (Fig. 3) and exhibit promotion of T_{reg} cell differentiation (Fig. 4).

Studies have shown that bacterial members of the clostridium, bacillus and butyrivibrio genera can produce and maintain high concentrations of butyrate in the small intestine and colon. Published works show that butyrate regulates the differentiation and maturation of DCs, and changes the cells' capability to capture and prime antigen-specific T cell activation [20,21]. These characteristics were confirmed in both butyrate-treated DC groups (Bu-DCs and RA-Bu-DCs) in this study, indicating the potential roles of butyrate in the gut. Outside the intestine, dietary fermentable fibre can also shape the immunological environment in the lung and influence the severity of allergic inflammation through SCFAs [22]. Interestingly, we found that CD103 and $\alpha_4\beta_7$ expression were only increased in RA-treated DCs (RA-DCs and RA-Bu-DCs), suggesting that CD103 induced transcription is

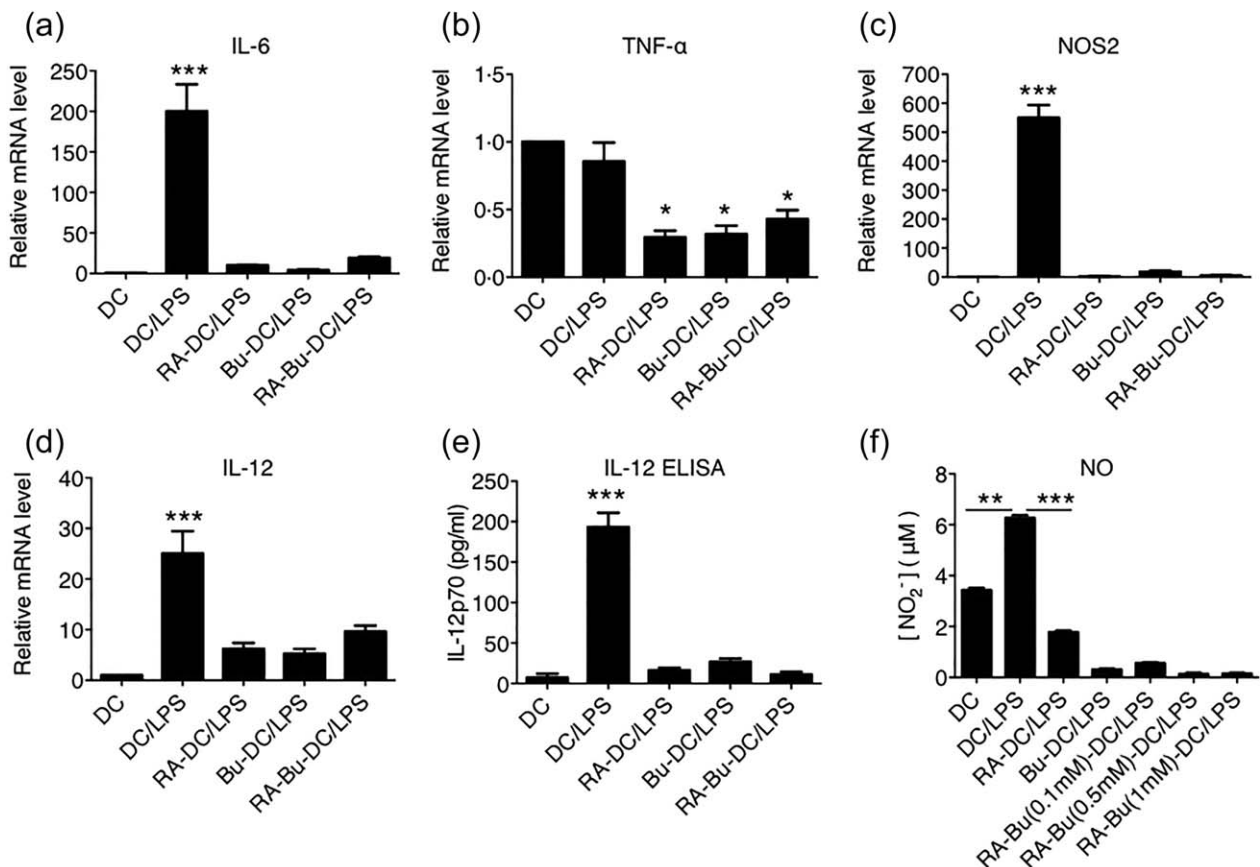


Fig. 3. Effect of butyrate on the cytokine profiles of dendritic cells (DCs). DC cells (5×10^5 /well) were re-seeded into 24-well plates and matured with 1 μ g/ml lipopolysaccharide (LPS) for 18 h. The relative mRNA levels of interleukin (IL)-6 (a), tumour necrosis factor (TNF)- α (b) and IL-12 (d) in DCs were quantified by quantitative polymerase chain reaction (qPCR) analysis. The supernatants of cultured DC cells were used to detect IL-12p70 protein using enzyme-linked immunosorbent assay (ELISA) (e) (c, f). The mRNA levels of nitric oxide synthase 2 (NOS2) in DCs (c) and NO in the supernatants (f) were quantified with qPCR and the Griess reaction, respectively. Data are shown as the mean \pm standard error of the mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

related closely to vitamin (retinol) metabolism, not to butyrate.

Certain mucosal DC subsets have reduced ability to prime CD4⁺ T proliferation than DC cohorts in peripheral tissue. Indeed, the priming ability of CD4⁺ T cell activation and proliferation was impaired seriously in all RA-DCs, Bu-DCs and RA-Bu-DCs (Fig. 4), indicating the regulatory functions of RA and butyrate on DCs. Studies have also shown that CD103⁺DCs can induce the generation of FoxP3⁺ regulatory cells from naive T cells in the presence of retinoic acid [16]. Other studies also revealed that RA stifles autoimmunity through up-regulating T_{reg} cell development, thus inhibiting the ongoing autoimmune disease [23]. We noticed that retinoic acid-treated DCs (RA-DCs and RA-Bu-DCs) had a stronger capability to induce T_{reg} cell differentiation, which was not inhibited significantly with butyrate treatment (Fig. 4). A series of contradictory statements exists in published data regarding the effects of butyrate on T_{reg} cells [24,25]. The exact role of SCFAs on T cell differentiation still needs more exploration.

In mammals, vitamin A is taken from food in the form of retinol, retinoic ester or beta-carotene. In the cell, retinol can be oxidized into retinal by retinol dehydrogenase (RDH) and then turn into retinoic acid by retinaldehyde dehydrogenase (ALDH) [26]. Accumulating data have shown that intestinal CD103⁺ DCs express retinal dehydrogenase (ALDH) 2 highly, which works as an enzyme to convert retinal to RA and then induce gut-homing T and B cells [27]. Furthermore, DCs can acquire high levels of retinoic acid-producing capacities in response to vitamin D₃ [28]. Here, our data also confirmed that the levels of RALDH were increased in RA-treated DCs (RA-DCs) (Fig. 4), indicating the importance of RA in the differentiation of immune cells in the gut. Unfortunately, the exact role of butyrate on ALDH is waiting to be demonstrated. Data from high-fibre feeding models showed that fibre-derived SCFAs, particularly acetate and butyrate, enhanced oral tolerance and protected against food allergy by enhancing ALDH activity in CD103⁺ DCs *in vivo* [28]. In contrast, our data showed that butyrate inhibited the levels of ALDH in DCs

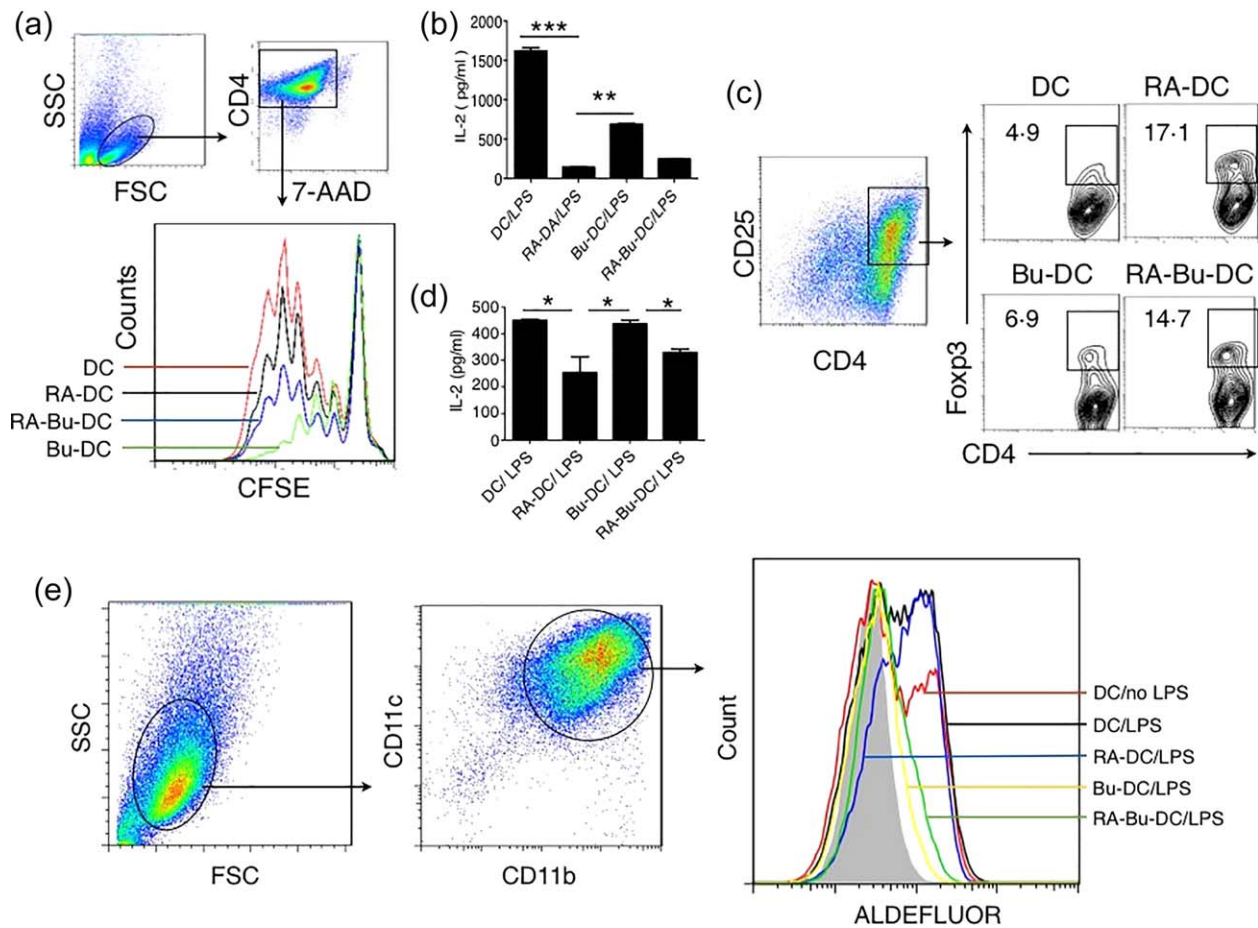


Fig. 4. Butyrate regulates the functions of retinoic acid (RA)-induced dendritic cells (DCs). (a,b) Butyrate regulated the capability of RA-DCs negatively in priming the proliferation of CD4⁺ T cells. DC cells were cultured with carboxyfluorescein succinimidyl ester (CFSE)-labelled ovalbumin (OVA)_{323–339}-specific CD4⁺ T cells at a ratio of 1 : 10 in U-bottomed 96-well plates. Cells were cultured further for 4 days. The divisions of 7-aminoactinomycin D (7-AAD)⁺CD4⁺ T cells were analysed with a flow cytometer (a), and the levels of interleukin (IL)-2 protein in the supernatants were detected using enzyme-linked immunosorbent assay (ELISA) (b). (c,d) Butyrate had little effect on regulatory cell differentiation. Naive CD4⁺ T cells were purified from the spleens of forkhead box protein 3 green fluorescent protein (FoxP3^{GFP}) × DO11.10 F₁ mice and cultured with OVA_{323–339} peptide-loaded DCs at a ratio of 1 : 10. After being cultured for 4 days, cells were collected. CD4⁺CD25⁺FoxP3⁺ regulatory T cells were identified with a flow cytometer (c), and the levels of IL-2 in the supernatants were detected using ELISA (d). The numbers in the graphs indicate the percentage of FoxP3⁺ cells in the CD4⁺CD25⁺ cells. (e) Butyrate inhibits aldehyde dehydrogenase in RA-DCs. The activity of aldehyde dehydrogenase (ALDH) in DCs was detected using ALDEFLUOR staining with a flow cytometer (e). Data are shown as the mean ± standard error of the mean. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. [Colour figure can be viewed at wileyonlinelibrary.com].

significantly. The research model used in the experiments may have caused this difference. Previous studies also showed that butyrate significantly induced IL-23 expression and promoted IL-17 secretion, indicating its contribution to increasing the severity of dextran sulphate sodium (DSS) colitis [29]. Interestingly, one colonic DC subset (CD103⁺CD11b⁺ALDH⁺) was identified recently, which displays a unique capacity to polarize T helper type 17 (Th17) cells [30]. Similar to the CD103⁺CD11b⁺ALDH⁺ DC subset, our data show that butyrate and RA treatment suppress the expression of CD11c and ALDH synergistically, but increase CD103 in RA-Bu-DCs. These data indicate the potential roles of butyrate and RA in the differentiation and function of this special colonic CD103⁺CD11b⁺ALDH⁺ DC subset.

Collectively, the data in this study suggest that CD103⁺ mucosal DC subsets, which control T cell homeostasis in the gut, are dependent upon the regulation of butyrate and RA, both individually and collectively.

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Disclosure

No conflicts of interest.

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